

SOP 9 – Seagrass Condition Monitoring

Version 1.0 (August, 2007)

The following table lists all changes that have been made to this Standard Operating Procedure since the original publication date. Any recommended or required changes added to the log must be complete and concise and promptly brought to the attention of the Project Leader. The Project Leader will review and incorporate all changes, officially complete the revision history log, and change the date and version number on the title page. For complete instructions, please refer to Instructions for Recording Revisions in SOP 13.

Revision History Log:

New Version #	Previous Version #	Revision Date	Author (full name, title, affiliation)	Location in Document and Description of Change	Reason for Change

Introduction

This SOP describes measurement of seagrass structural characteristics used as early indicators of estuarine condition in response to nutrient enrichment. The goal here is neither to evaluate the overall condition of seagrass resources within NPS Northeast Region (NER) coastal parks, nor to detect early loss of seagrasses on a park-wide scale. The sampling design is inappropriate to address these questions. Instead, this SOP describes an efficient approach to using targeted seagrass monitoring as an indicator of estuarine nutrient enrichment. The monitoring protocol is based on population- and shoot-based measures of seagrass condition, as well as important ancillary variables that influence seagrass responses to nutrient load. Procedures are derived from the SeagrassNet protocol for global seagrass monitoring (www.seagrassnet.org), a program designed to investigate and document the status and trends of seagrass resources around the world. The program was originated in 2001 in the Western Pacific, where 19 monitoring sites were established in seven countries. Since then, many more SeagrassNet sites have been established around the world. Two of the U.S. sites were established at CACO during development of this SOP. We have adapted SeagrassNet methods (Short et al. 2004) for use in NER coastal parks to accommodate regional characteristics of seagrass ecosystems and logistical constraints in implementation. The NPS procedures for monitoring seagrass condition are consistent with the global SeagrassNet program in fundamental aspects of sampling design, measures, and sampling methods, so that NPS data may contribute to the global SeagrassNet database. Use of methods compatible with SeagrassNet provides a global framework for interpretation of NPS monitoring data.

SeagrassNet monitoring is based on repeated measurement of permanent quadrats located along three 50-m transects that are positioned parallel to shore and along a depth gradient. The sampling objective is not to describe trends in condition of an entire seagrass bed, which would

require a large sampling investment, but rather to describe changes in seagrass characteristics over time at locations that are representative of the seagrass depth range. In general, seagrass responses to nutrient enrichment will be detected first at the deepest locations of existing beds (cf. Background and Objectives, Seagrass Condition). Consequently, comparisons of trends in seagrass characteristics between shallow and deep locations can be helpful in forecasting seagrass declines. This approach to monitoring seagrass condition provides high resolution information that complements large-scale measurement of seagrass distribution (SOP 8 – Submerged Aquatic Vegetation Mapping). Random placement of sampling quadrats along transects ensures unbiased measurements at each transect location, and repeated measurement of specific quadrats provides the maximum ability to detect changes over time.

Sampling is conducted at extreme low tides to minimize water depths. Snorkeling ability is required to sample some of the quadrats; although use of SCUBA would facilitate sampling the deepest quadrats, the methods presented here have been designed for implementation with snorkeling alone. The following methods are drawn extensively from the SeagrassNet monitoring manual (Short et al. 2004), which are based on a compilation of seagrass research techniques for global application (Short and Coles 2001). NPS personnel involved in monitoring seagrass condition should refer to the SeagrassNet manual for an overview of the global program and more detailed descriptions of methods where noted.

One notable departure from SeagrassNet protocols is the omission of short-term continuous light monitoring within the study site using unwiped logging light sensors. Extensive PAR monitoring is already prescribed for NER estuaries using both spatial surveys and high-quality continuous monitoring equipment. These approaches provide reliable data on PAR that is relevant to seagrass condition, making additional deployment of light sensors unnecessary.

Equipment and Supplies

The following equipment and supplies are used for within-bed seagrass monitoring:

- plant press and herbarium supplies (acid-free herbarium mounting cards, museum-quality mounting glue, herbarium specimen labels)
- 50-m measuring tape (3)
- 100-m measuring tape (2)
- compass
- 0.25 m² (50 cm x 50 cm) stainless-steel quadrat with washers welded in the middle of each edge as guides for subdivision rods
- 75-cm long orange fiberglass rods with tapered ends for subdividing quadrats into 4 0.0625-m² sections (2)
- 0.25 m² photo quadrats made of ½-inch CPVC
- long orange fiberglass rods for temporary station markers
- flagging tape
- cable ties, 8-inch black UV-stabilized
- side cutters for clipping cable ties
- meter stick
- digital land camera
- digital underwater camera

- floating plexiglass view frame, 60 cm x 60 cm (plexiglass attached to 5-cm deep wood frame that is covered with foam rubber)
- percent cover standard
- GPS unit, WAAS enabled
- permanent station marker (32-inch helical screw anchor, 15)
- crow bar, thick dowel, or other tool for installing screw anchors
- buoys (6)
- recording thermometer, Stowaway[®] TidbiT[®] or iButton[®] temperature logger (2)
- optic base station for launching TidbiT[®] temperature logger **OR**
- Blue Dot Receptor, 1-wire to RS-232 Adapter, optional USB to 1-wire adapter, and iButton Viewer software for equipment for launching iButton[®] temperature logger (see SeagrassNet manual for instructions)
- salinity meter or refractometer
- small bottles for salinity samples (3)
- plastic or stainless steel corer, 6.7-6.8 cm internal diameter, with end cap or stopper
- 20cc syringe sediment sampler, with rubber stoppers to seal
- dive slate printed with data sheet, with attached pencil
- mask, snorkel, booties, wet suit, weight belt (plus dive gear if using SCUBA)
- nylon sample-bags, 1/16-inch mesh (36)
- insulated chest
- plastic bags (gallon-3; quart-12)
- plant sorting trays
- forceps for sorting plant samples
- glass microscope slides
- drying oven
- paper bags for drying plant samples (small Plain Kraft “merchandise bags”)
- data sheets copied on waterproof paper for Cross transect A, Cross transect B, Cross transect C, and Light and Temperature Sensor Deployment Log (Appendix F)
- pencils, permanent markers
- clip board
- underwater video drop camera (see Distance to Deep Seagrass Edge and Last Shoot for recommended customization)

Establishing the Monitoring Station

The site selected for monitoring seagrass condition at a given park should be representative of the park’s seagrass resources. The site should reflect the depth range where seagrasses are typically found, and should not be exceptional in any characteristic. Ideally, seagrass distribution at the site should be fairly even, without large bare patches or physical disruptions such as tidal channels or sand bars. Homogeneity of the seagrass bed reduces long-term variability in sampled parameters and enhances the likelihood of detecting responses to nutrient enrichment. One of the most important considerations in site selection is accessibility; monitoring personnel must be able to access the site easily and safely by land or by boat. To ensure long-term monitoring of effects of estuarine nutrient enrichment, the site should be free of obvious large human impacts (e.g. dredging, boat moorings) that could disturb or eliminate seagrass habitat at that location.

Establish a transect perpendicular to the shore at the site determined to be the most appropriate for monitoring. The transect should begin about 1 m inside the continuous seagrass meadow. Mark the beginning of the transect with a temporary marker (fiberglass rod flagged with fluorescent tape); this will be Station A. The global SeagrassNet protocol calls for the seaward end of the transect to be located approximately 1 m inshore of the deep edge of the continuous seagrass bed. However, for NPS implementation, sampling logistics must also be considered in locating the transect's seaward end, as the deep edge of the seagrass beds in this region is often too deep to be sampled by snorkeling alone. If personnel are qualified and authorized to use SCUBA, then the seaward end of the transect can be located near the deep edge of the seagrass bed. If sampling will be done by snorkeling, then the deep end of the transect should be located at the deepest point that is comfortably and safely accessible by snorkeling (typically waist- to chest-deep at mean low water). Mark the end of the transect with a temporary marker (fiberglass rod or weight with rope and float attached, depending on the depth); this will be Station C. Identify a location on the transect that is mid-depth between Stations A and C and mark it with a temporary marker; this will be Station B. Stations A, B, and C should now fall on a straight line that is perpendicular to the shore.

Three 50-m cross transects will be established along the shore-normal transect delineated by the temporary markers. Establish cross-transect A at the shallow location that was identified about 1 m inside the continuous meadow. Screw a permanent marker (screw anchor) into the sediment so that only the "eye" at the top of the shank remains above the sediment. This is the center point for cross-transect A (i.e. position A25, at a distance of 25 m along the 50-m cross transect). Use a tape measure to lay out the cross-transect so that it is 50 m long, roughly parallel to the shore, with the marker A25 at the center and following the same depth contour as closely as possible along the entire length. This will require three people, one stationed at the center and one on each end, to be sure the cross transect is straight, exactly 50 m long, with A25 at the exact center. Install permanent screw anchors at each end of the cross transect (A0, located at the left end of the cross transect when facing the sea, and A50, located at the right end of the cross transect when facing the sea). Record the GPS positions for the three permanent cross-transect markers and record the compass bearing of the cross transect from A0 to A50. Note that future sampling will occur by securing a tape measure to the permanent screw anchors, and locating permanent quadrat locations by their distances along the tape measure. In high energy environments it is advisable to install additional screw anchors for tape measure attachment midway between A0 and A25, and midway between A25 and A50, to reduce potential bowing of the tape measure along the 50-m cross transect. Short (ca. 50 cm) lengths of polypropylene line can be attached to the exposed end of the screw anchors as visual aids for relocation. Finally, install an extra screw anchor at each end of the cross transect, in line with the cross transect and 1 m away (to the outside of the cross transect). These screw anchors can be marked with buoys to assist in relocating the cross transect; extra screw anchors are installed for buoy attachment so that attention is not drawn to the actual cross-transect markers. Record the GPS positions of the extra screw anchors for relocation purposes as well. Repeat this procedure for cross-transects B and C, at previously identified Stations B and C. Note that when all three cross transects have been established, their compass bearings should be the same.

Sampling Frequency

Seagrass monitoring should occur quarterly (April, July, October, January) during at least the first year of monitoring to establish baseline seasonal trends in condition measures. If possible, two years of quarterly monitoring provides information on annual variability in these trends. The minimum monitoring frequency thereafter is once per year, although continued quarterly sampling is advantageous and advisable if resources allow. Global SeagrassNet monitoring is conducted quarterly in perpetuity. Annual monitoring of seagrass condition in NER coastal parks should occur in July, which is at or near the time of maximum seagrass biomass in this region (Roman and Able 1988, Kinney and Roman 1998, Short et al. 1993). July sampling also coincides with one sampling period of the global program, ensuring that NPS data are compatible with SeagrassNet and can contribute to the global database for interpretation within a broader context.

Re-establishing the Cross Transects for Sampling

Monitoring should occur in the shallowest tides possible during daylight hours. Use reliable tide tables (e.g. NOAA tables at http://co-ops.nos.noaa.gov/tide_pred.html) to identify predicted daytime low spring tides for sampling. Depending on the site logistics and number of personnel, it may require two low tides to sample all three cross transects. Monitoring personnel should arrive on site 1.5-2.5 hours before the predicted time of low tide to allow sufficient set-up time; sampling should begin as soon as water depths are workable, usually within 1-2 hours before low tide.

After locating the monitoring site, find the ends of the cross transects using buoys and/or GPS. Mark the ends of shallow transects with fiberglass rods and flagging tape to make transect work easier. Clean any algal growth or other fouling material off the permanent markers. Lay out a 50-m tape measure at all cross transects to be measured that day. Attach the measuring tape at “zero” to the transect screw anchor at the left side of the cross transect when looking at it facing the sea. Stretch the tape through the middle screw anchor (the Station marker) and attach it to the transect screw anchor at the right side of the cross transect (facing the sea). Remember that the screw anchors that mark the ends of the cross transects are 1 m toward the transect from the buoyed site markers. It is essential that the tape measure be stretched taut between the ends of the cross transect, as distances are used to relocate permanent quadrats.

Over five-year to decadal time scales, the edge of the seagrass meadow at permanent station A or C may shift. If either the shallow or deep edge of the bed has shifted so that the entire cross transect no longer has seagrass, establish a new Station A' or C' approximately one meter inside the new edge of the continuous bed. Conduct the sampling from cross transects based on these new locations and note the change on the data sheet. Measure and record the distance and depth differences from A to A' or C to C'. Record on the data sheet that there is no seagrass present on the original cross transect.

Station Measures

The following observations and measurements are taken during each monitoring event to characterize the environmental conditions at the depth stations.

Monitoring Event Details

General information about the monitoring event is recorded at the top of each cross-transect data sheet. Record the park unit, site name, sampling date, local time that sampling was initiated, personnel, weather (sky and wind conditions), predicted time and height of low tide with location of NOAA tide station used for reference, and any noteworthy comments (special conditions or observations).

Temperature

Water temperature is measured continuously at the shallow (A) and deep (C) monitoring stations. Temperature loggers are deployed and retrieved during each logging event. The duration of an individual logging interval is determined by how frequently monitoring occurs. Temperature is measured and logged with the Onset Stowaway TidbiT[®] temperature logger or the iButton[®] temperature logger. The iButton[®] logger is less expensive, but due to production limitations may be unavailable when needed. Although the Stowaway TidbiT[®] loggers are more expensive, we have never found them to be backordered. Instructions below relate to the Stowaway TidbiT[®] logger. Detailed instructions for launching and downloading the iButton[®] logger is provided in the SeagrassNet manual (Short et al. 2004).

Launching the Temperature Loggers: Stowaway TidbiT temperature loggers must be launched before deployment using Onset's BoxCar[®] (basic Windows[®] package for logger management) or BoxCar[®] Pro (includes advanced graphing and analysis functions) software. Connect the Optic Base Station to your PC using the PC interface cable provided with the software. Note that the cable is equipped with a 9-pin RS-232 female connector for direct connection to the computer's serial port. If your computer has only USB ports then a USB Serial adapter (available from Onset[®]) will be necessary to connect the Optic Base Station to the computer. Record the date and time of launching on the Temperature Sensor Deployment Log.

Follow the steps below to launch the temperature loggers.

- Open the BoxCar[®] software package on your computer.
- Connect the first TidbiT[®] onto the TidbiT[®] coupler attached to the Optic Base Station.
- Select "Launch" from the Logger menu. A dialog box will appear.
- Enter the appropriate logger label in the Description field. The label should include the 4-letter park unit code, site name, and station label (A or C).
- Select a 30-minute logging interval.
- Select "Temperature (°C)" as the measurement unit.
- Be sure that "Wrap around when full" is not checked (appears as an "Advanced Option" in BoxCar[®], and at the bottom of the dialogue window in BoxCar[®] Pro). This option instructs the logger to continue logging when the memory is full by overwriting the oldest data. If logger retrieval were delayed, this could result in loss of meaningful data. If this option is not selected the logger will stop logging data when the memory is full.
- Select "Delayed Start"

- Enter the date and time to begin logging. Entering a time shortly before you plan to deploy the logger avoids accumulation of meaningless data that would have to be culled from the record.
- Ensure that Multiple Sampling is “Off”. (Multiple Sampling causes the logger to make multiple measurements during the logging interval and store only the minimum, maximum, or average of the readings).
- Select “Start”. A dialog box will appear to ensure that you want to launch the logger, as this will erase all previously collected data from the logger. Confirm that all data on the logger have already been downloaded and saved to a computer file before selecting “Yes”.
- Disconnect the logger from the interface cable before selecting “OK”.
- The green light should blink every four seconds, indicating that the logger has been launched and is waiting out the time delay. When logging has begun the green light will blink every two seconds.
- Repeat this process for the second temperature logger.
- Place the logger in a thermally stable environment and, using NPS traceable thermometer, measure and record the room temperature along with the exact time and date. These will be compared to the logger reading upon its recovery.

Installing and Retrieving the Temperature Loggers: The temperature loggers are attached to the permanent screw anchor placed at the zero end of cross transects A and C. Deploy the temperature logger by securing it to the screw anchor with a cable tie just above the sediment-water interface. This position ensures that the sensor is not exposed to air unless the seagrass bed is completely drained. Every time the station is monitored, each temperature logger is removed and replaced with a freshly launched logger. Place the retrieved logger in small plastic bags labeled with the logging location and record the date and time of deployment and retrieval on the Temperature Sensor Deployment Log.

Salinity

Salinity is measured once during the monitoring event at the center permanent station marker (A-25, B-25, and C-25). Salinity should be measured close to the same tidal stage at all three stations when the entire site is submerged. Salinity can be measured *in situ* by transporting a salinity meter equipped with a submersible probe to the station (e.g. YSI 30 System instrument) or by collecting a small water sample for subsequent measurement. Regardless of approach, it is important that salinity is measured from a well-mixed area within the seagrass canopy rather than from the water above the canopy. To use a salinity meter *in situ*, lower the salinity probe into the seagrass canopy before recording the salinity. Alternatively, collect a water sample from the right depth by submerging a collection bottle with the lid on into the seagrass canopy, removing the lid to fill the bottle, and replacing the cap before raising the bottle. In this latter case, the salinity should be read as soon as possible after the sample is collected, using either a salinity meter or a refractometer. Instructions for proper use of a refractometer are provided in the SeagrassNet manual (Short et al. 2004). Whether using an electronic salinity meter or refractometer, the accuracy of the instrument should be checked in standard seawater or conductivity standard at least once for each monitoring event (season).

Cross Transect Measures

The following measurements are made to characterize the structure of the seagrass bed and the environmental conditions along the cross transects.

Distance to Shallow Seagrass Edge and Last Shoot

Using a 50- or 100-m tape measure as needed, measure the distance onshore from the left (A0), center (A25), and right (A50) cross-transect positions perpendicularly to the limit of the seagrass bed. Two measures are taken to define the extent of the seagrass growth at the time of sampling. Distance onshore to edge of bed (m) is measured as the distance to the farthest point where shoots are less than or equal to one meter apart. Distance onshore to the last shoot (m) is measured as the distance to the most inshore shoot from cross transect A. Record these distances on the appropriate cross-transect data sheet in meters to the nearest tenth of a meter. If the edge of the seagrass has contracted inside the position of the cross transect, record the distance as negative. In beds with dynamic edges, it may be helpful to sketch a diagram of the site on the back of the data sheet, specifying where the inner and outer edges of the meadow are located.

Distance to Deep Seagrass Edge and Last Shoot

Conceptually, these measurements are analogous to those described in the previous section. The goal is to record the deep edge of the bed by measuring its distances from the deep permanent cross transect. Unlike the shallow edge, however, these distances may be quite long and measuring them with a meter tape will not be feasible. Instead, distances are measured using WAAS enabled GPS and underwater videography. Distance are measured offshore from the Left (C0), Center (C25) and Right (C50) reference marks of cross transect C. Note that only the deep edge of the bed is measured, not the last shoot.

It is assumed that a small boat will be used for this task, so consistent methods for navigating the craft along the desired offshore course become important. To identify the proper offshore course, use a computer graphing application to plot the latitudes (Y-axis) and longitudes (X-axis) of reference marks A0, B0, and C0. Use the coordinates already collected for these points, converting them into units of decimal degrees. Fit a least squares linear regression to these points and record the slope of this line. Repeat this process for reference marks A25, B25 and C25; and then again for A50, B50, and C50. Calculate the average slope of these three offshore trajectories. Note that these calculations assume a flat earth, a reasonable assumption for all distances anticipated during application of this SOP.

Using the average slope of these offshore trajectories, project a line from reference mark C0 and calculate points that fall along this line that are no farther than 25 m apart from each other. The coordinates for these points, listed in order, become “waypoints” along a “route” that can be piloted using navigation features commonly found on even the most inexpensive GPS units. Repeat the process to create offshore navigation routes for C25 and C50 using the same average slope from the previous paragraph.

Using a GPS with these three programmed routes, it is a relatively simple matter to navigate a small boat offshore from C0, C25 and C50. The seagrass bed is observed using an underwater drop camera, through which the deep edge of the bed is identified. Once identified, latitude and longitude of this edge are recorded, so that offshore distance can be calculated between it and the

reference mark. This last step sounds much easier than it is; patchiness and holes in the bed make it difficult, and the bed edge is always passed before it is recognized. To make this task easier, and to establish a permanent visual record of the bed, we recommend using an underwater video drop-camera with the ability to record the video stream and integrated overlay of GPS data (latitude, longitude, date, time, speed, heading). This way data can be collected rapidly in the field, then the bed edge position evaluated more carefully back in the laboratory. For pilot testing we assembled one such camera system (Figure 25) for a total cost of under \$1600 (Table 17). The selected camera also included on-screen data for temperature and depth.



Figure 25. Screen capture from underwater video taken at Cape Cod National Seashore using the described video equipment.

Table 17. Components and prices for a waterproof drop camera system with integrated waterproof deck unit consisting of a display, a digital recording device, a WAAS enabled GPS unit, and a video overlay unit to project GPS data onto the recorded video.

Aqua-Vu 120' DT video camera	\$632
Black Box Camera GPS Video Overlay Controller	\$126
Sony DCR-TRV460 Digital-8 HandyCam	\$420
Garmin eTrex Legend GPS	\$200
Waterproof Pellican Case #1450	\$95
Garmin GPS Marine Mount	\$36
CableClam waterproof bulkhead cable pass-through	\$20
Plexiglass window and other miscellaneous parts	\$70

Depth

Water depth is measured at the 0-, 25-, and 50-m positions of cross transects A, B, and C. Depth must be measured at each of the nine locations as close to the same time as possible, when the entire site is submerged. If any of the cross transects are intertidal, wait until all are submerged before measuring depth. Recording the depths of all locations at approximately the same time allows determination of the relative depth differences among sampling locations without introduction of error from tidal variation between measurements. Measure the depth from the top of the substrate at each location using a depth gauge (e.g., meter stick, long pole marked with depths, weight on a rope marked with depths, etc.). Record the depth and time on the datasheets.

Surface Sediment Observation and Sample

Assess the sediment at the 0-, 25-, and 50-m positions of each cross transect by feeling the texture of the top two centimeters of substrate. Classify the sediment as one of the following: mud, fine sand, sand, coarse sand, shell, gravel, or rock. Record the sediment type in the three appropriate fields on each cross transect data sheet.

Sediment samples are collected from the 0-, 25-, and 50-m positions of each cross transect with a 20-cc syringe corer (plastic syringe with graduated tip cut off flush with the zero volume mark). Place the cut off end of the syringe barrel at the sediment surface, hold the plunger at a constant height at the sediment surface, and push the barrel into the substrate. Gently extract the sediment-filled syringe from the substrate and cap the cut off end with a rubber stopper. Empty the entire contents of the syringe into a small plastic bag labeled with the sample location and

date. Close the bags tightly, keeping as little air in the bags as possible. Indicate with check marks on the data sheets that sediment samples were collected.

Sediment samples should be transported to the laboratory in an insulated chest. Samples should be analyzed for grain size distribution (gravel, sand, and silt+clay fractions) and carbonate, organic matter, and organic carbon content. Analytical methods are described by Erftemeijer and Koch (2001). Although these analyses may be conducted in-house, in most cases a contract laboratory will be used. Samples may be stored at 2°-5° C in a refrigerator for several days or frozen at -20°C for up to one year.

Voucher Specimen

Seagrass voucher specimens are collected at each monitoring event for future reference. Collect several individual ramets in the vicinity of the center of each cross transect (A25, B25, and C25). Select representative specimens and ensure that you have all the plant parts, including leaves, rhizomes and roots. Include plants with reproductive structures when present. Only a small sample of 3-5 complete ramets is needed. Place the seagrass sample inside a large, labeled, plastic bag with a small amount of seawater. Indicate with a check mark on the data sheets that voucher specimens were collected.

The voucher specimen should be pressed as soon as possible after collection. If it is going to be more than two hours before the sample is pressed, it should be rinsed in fresh water and refrigerated to prevent any decomposition. Do not refrigerate longer than two days. Rinse the sample in fresh water before pressing and carefully clean of debris, large epiphytes, or sediment particles. Retain the best two specimens for pressing. Lay out each specimen on an acid-free herbarium card, spreading leaves and roots to make each part of the specimen distinctly visible. Fill out specimen labels with the site information, including park code, site name, cross transect, latitude/longitude, date of collection, depth, percent cover, other species present, collector, and comments (such as stage of flower development for reproductive shoots, DeCock 1980). Place another clean sheet of herbarium paper over the specimen and layer within newspaper, blotters, and cardboard ventilators within a standard plant press. Tighten the press and allow specimens to dry in a dry, well ventilated place for a minimum of two weeks. For best results, replace the newspaper after two to three days.

Mount one specimen and label on the herbarium card with archival quality glue and maintain with local park collections for reference. Send the other specimen, with its label, to SeagrassNet for archiving (see SeagrassNet manual for contact information).

Quadrat Measures

The following measures of seagrass abundance and shoot morphometry are monitored at 12 permanent locations along each cross transect. The sampling locations were selected randomly and are listed on the appropriate cross-transect data sheets (see attachment). Sampling occurs within 0.25 m² (i.e. 50 cm x 50 cm) quadrats that are subdivisible into subquadrats. The exact sampling locations are determined by placing the sampling quadrats on the beach side of the tape measure with the bottom right corner of the quadrat on the sampling distance. For example, the random location for quadrat 1 on cross-transect A is 2 m along the cross transect tape measure, so the quadrat is positioned from 2 m to 2.5 m on the beach side of the tape. Note that since sampling occurs on the beach side of the tape, monitoring personnel should always walk on the seaward side of the tape to avoid disturbance to sampling areas. Any unattached algae or large mobile animals should be removed from the quadrats before sampling. For simplicity, the methods below instruct field personnel to record measurements on the cross-transect data sheets. In practice, when using snorkel or scuba gear to monitor submerged quadrats, it is easier to record all the data on a dive slate upon which a data grid of data fields identical to the data sheet has been printed. The dive slates should be photocopied, and quadrat data transferred to the cross-transect data sheets or entered into computer files and checked for accuracy as soon after sampling as possible.

Photographs

Photographs are taken of seagrass quadrats as permanent visual records of seagrass condition. They also can serve to verify field data. The quality of quadrat photos is highly dependent on weather conditions. Under ideal conditions (sunny and calm), all quadrats should be photographed. However, if sampling conditions are extremely overcast or windy and wavy, obtaining informative photographs can be difficult and time consuming. Under these conditions, it is acceptable to photograph only three quadrats per transect. These mandatory photo quadrats are those occurring at the beginning, near the middle, and at the end of each transect, and are identified as “required” on the data sheet (with all other quadrats identified as “optional” for photo purposes).

Photos should be taken either before or considerably after other quadrat sampling is done to avoid turbidity created by walking, swimming, or sampling in the area. It is important to either include a quadrat label in the photo or to keep careful field records relating photo numbers to quadrat locations. Be sure that the camera’s internal calendar is set to the correct date so that photo file details include this information. Photographs should be taken from as vertical an angle as possible and include the entire quadrat frame and the tape measure. Use the CPVC 0.25m² quadrat for improved frame visibility in the photo. Ideally, photos should be taken either when the seagrasses are exposed at the surface (land camera) or when the seagrasses are fully submerged (underwater camera). Depending on the tide height, when using an underwater camera it may be impossible to capture the entire quadrat frame in a single vertical photo. In this case, individual vertical photos should be taken of sub-sections of the quadrat clockwise from the lower right (i.e. lower right, lower left, upper left, and upper right sub-sections of the quadrat), and a fifth photo should be taken from an oblique angle to capture the entire quadrat frame. Quadrats can be photographed at intermediate depths using a land camera and a plexiglass viewing frame that is floated on the water over the quadrat. A thin film of water spread on the top of the plexiglass prevents reflection, facilitating high-quality photos of the seagrasses under

the viewing frame. Mark the “photograph” box under the appropriate quadrat on the datasheet for all photos that are taken. The photo number can be recorded on the data sheet as well.

Percent cover, Shoot Density, Reproductive Shoots

Record the percent cover of the seagrass canopy within each entire quadrat, using a laminated percentage cover photo guide as a reference (provided in the SeagrassNet manual). Record the seagrass species within the “percent cover” data field. SeagrassNet procedures for measuring percent cover account for multiple, co-occurring species found in tropical seagrass beds; total seagrass cover is recorded, as is the percent cover of individual species. In general, NER coastal parks with extensive seagrass resources support single-species beds (*Zostera marina*, eelgrass; or *Ruppia maritima*, widgeon grass) and the data sheet associated with this SOP assumes that single species beds are being monitored. Eelgrass and widgeon grass do co-occur in the southern parks in the region (FIIS and ASIS). If mixed-species beds are most representative of the seagrass resources at a particular park and the monitoring site includes more than one seagrass species, then percent cover of total seagrasses as well as that of each individual species should be recorded (note that the data sheet would need to be modified accordingly by adding additional rows for “total seagrass” and a second species). In this case, note that the percent covers of individual species must sum to the percent cover recorded for total seagrass.

Shoot density is determined by direct counts of all shoots rooted within the entire permanent 0.25 m² quadrat or within a 0.0625 m² subquadrat, depending on percent cover of seagrass within the entire quadrat. Shoot density should be determined using the entire quadrat if percent cover is low (less than or equal to 25%) or if shoot distribution within the quadrat is highly clumped (i.e. the majority of shoots are rooted within a subsection of the quadrat). Conversely, shoot density should be determined using a 0.0625 m² subquadrat if percent cover is high and shoots are fairly homogeneously distributed. If needed, form density subquadrats by threading the fiberglass dividing rods through the guides at the centers of opposing quadrat sides. This yields four subquadrats of 0.0625 m² each. For density measurement, select the subquadrat in which percent cover most closely approximates that of the entire quadrat. Count the total number of shoots rooted within either the entire quadrat or the selected subquadrat, as appropriate, and record this count within the “density” data field. Record the location of the shoot count in the “source” data field as the entire 0.25 m² quadrat (E= entire), or one of the four 0.0625 m² subquadrats (identified when facing shore as TL= top-left, TR= top-right, BL= bottom-left, BR= bottom right). Should preliminary data show that shoot counts in excess of 3000 / m² (188 / 0.0625 m² subquadrat) are regularly encountered, then it would be acceptable to use smaller, 10 cm x 10 cm subquadrats (cf. Duarte and Kirkman 2001). In this case quadrat frames would need to be modified with additional divider guides.

Check the quadrat to determine if reproductive shoots (with flowers or fruits) are present. For eelgrass, count the number of reproductive shoots occurring in the entire quadrat and record this number on the data sheet within the “Flowering Shoots” field. For widgeon grass, count the number of flowering shoots within the subquadrat area used for density measurement. Note the area of the sample (field count per ¼ m² or 0.01 m²) on the data sheet.

Grazing, Epiphyte Cover, Wasting Index

The degree of grazing, epiphyte cover, and wasting disease (eelgrass only) present in the quadrat is classified using a visual assessment of ten terminal, vegetative shoots. Wasting disease causes characteristic black lesions that spread longitudinally along eelgrass leaves. Burdick et al. (1993) developed a Wasting Index (WI) based on the relative amount of necrotic tissue on the most infected eelgrass leaves in a sample. We adapted the WI for rapid field assessment by classifying the percentage of infected leaf area into one of three categories: trace (T, 0-1%), low (L, 2-30%), or high (H, 31-100%). We selected the threshold between “low” and “high” categories based on data presented by Burdick et al. (1993) suggesting that as the WI exceeds about 30%, wasting disease in an eelgrass population progresses into a phase of rapid infection and disease spread. A similar, cover-based index for estimating *in situ* epiphyte biomass in seagrass beds has been proposed (Miller and Virnstein 1995), although ecologically relevant thresholds have not been defined. We adopted the same T, L, and H classification scheme for assessing grazing and epiphyte cover for sampling convenience.

Examine the most infected leaf of each of ten terminal shoots and classify the percentage of infected leaf area as trace, low, or high as described above. Burdick et al. (1993) provide a visual guide to different percentages of infected leaf area. Keep a mental tally of the number of leaves falling into T, L, and H categories. Record the overall average category in the “Wasting Index” field on the data sheet. This value is not a true mean of continuous data, but rather is used as an objective measure representing a compromise between field efficiency and quantitative assessment. Measure and record the degree of epiphyte cover by similarly classifying epiphyte coverage of the most epiphytized leaf, and grazing by classifying the degree of grazing evidence (scraped epidermis, bites out of leaf margins, clipped leaf tips) over the entire shoot, for each of ten terminal shoots. With practice, WI, epiphyte cover, and grazing can be assessed simultaneously in a short amount of time per quadrat.

Canopy Height

Seagrass canopy height is measured by grabbing a clump of plants rooted within the quadrat, extending the leaves (without uprooting the plants) to their maximum height, ignoring the tallest 20% of the leaves, and measuring the distance from the sediment to the top of the remaining 80% of the leaves. Use this technique to measure the canopy height in four locations within the quadrat (one clump of plants in the middle of each quarter of the quadrat); record the average canopy height on the data sheet.

Seagrass Biomass

Field Collection: If percent cover of a quadrat is zero, no biomass core is taken. For all quadrats with seagrass present, select an area for sampling that is 0.5 – 1.0 m landward of the quadrat and is of the same canopy height as inside the quadrat. Take a sample using a plastic (0.0035 m²) or stainless steel (0.0037 m²) corer, depending on the hardness of the substrate. Place the corer over the area to be sampled, ensuring that all of the leaves originating inside the area to be sampled are inside the corer. Rest the corer on the substrate and run your finger around the perimeter; remove leaves from the corer that are rooted outside the perimeter, and insert any remaining leaves into the corer that are rooted inside the perimeter. It is very important to position the plants inside the corer so that they are not cut during sampling. Push the sharpened edge of the corer vertically into the substrate to a depth of about 10 cm below the depth of the

seagrass rhizomes. For eelgrass in NER coastal parks, a core depth of 15 cm is adequate to ensure collection of all root material; widgeon grass rhizomes lie directly beneath the surface, so a core depth of 10-11 cm is sufficient. Place a cap or stopper on the top end of the corer and gently pull it out of the substrate. Seal the bottom of the corer with a cap or your hand as it is removed from the sediment. Empty the entire contents of the corer into a nylon mesh bag labeled with the quadrat number. Rinse the sample gently to remove as much sediment as possible. Record that a biomass sample was taken and the corer size on the data sheet. Place all samples from a single cross transect into a large mesh bag labeled with the cross-transect name. Samples should be transported to the laboratory in an insulated chest.

Laboratory Procedure: Samples should be processed as soon after collection as possible, ideally on the same day. If samples cannot be processed immediately, rinse the samples in fresh water and store in a refrigerator for up to two days. All samples should be rinsed in fresh water to remove salt before processing. Empty the sample into a sorting tray and remove all the intact shoots and all the live below ground material; loose, unattached leaves are assumed to have been included in the corer inadvertently and are not retained in the sample, but all living roots and rhizomes are retained. Clean the retained material of any remaining sediment by rinsing in fresh water, and scrape epiphytes off the leaves using a glass microscope slide. Count the number of leaf meristems (the base of the shoot where the leaves attach to the rhizome) in the sample and record on the data sheet in the “Number of meristems in biomass core” field. Separate the shoots into the following sample components for biomass determination: 1) leaves, including all intact material connected to a rhizome (green leaves as well as any dead attached leaves); 2) sheaths, including only living sheaths attached to rhizomes without blades still attached (i.e., sheaths with blades attached are included in the leaf component, and black, dead sheaths without blades attached are excluded); 3) roots and rhizomes, including all the living rhizomes (whitish to brown in color) and roots. Place each clean biomass component in separate paper bags labeled with the sample date, site abbreviation, cross-transect letter, quadrat number, and plant part. Dry the samples in a drying oven at 60° C for 48 hours or until completely dry. Cool the dried samples in a desiccator and weigh on an electronic balance in grams to an accuracy of three decimal places. Record the sample identification and weight on a data sheet or Excel spreadsheet.

Quality Assurance Measures

Seagrass condition measures involve numerous counts (shoot densities), measurements (heights, depths, distances), categorizations (wasting disease, grazing and epiphytization indices) and classifications (percent cover). These are often taken under challenging conditions by a minimum of two separate observers. In order to quantify intra and inter-observer variability, a minimum of 10% of each observer’s quadrats measures shall be duplicated, and a minimum 10% of all quadrat measures shall be replicated by all observers. Replication shall be performed in any logistically reasonable fashion that achieves the prescribed 10% for each monitoring event (site and season); however duplicate assessments of a quadrat by any observer must be separated in time by measurements at other quadrats (must not be sequential).

Post-sampling Procedures

Downloading Data from the Temperature Loggers

The following instructions pertain to Onset Stowaway TidBiT[®] temperature loggers. Detailed instructions for downloading temperature data from iButton[®] temperature loggers are provided in the SeagrassNet manual.

- Prior to initiating a download, allow all sensors to thermally equilibrate to room temperature, then record room temperature using an NBS-traceable thermometer. This value must be compared to logged values for quality assurance purposes.
- Open the BoxCar[®] software package on your computer.
- Connect the first TidbiT[®] onto the TidbiT[®] coupler attached to the Optic Base Station.
- Select “Readout” from the Logger menu. A dialog box will appear that says “Connecting...” and then “Stowaway found”
- The “Offload” dialog box will appear and signal that the data are downloading
- The “Disconnect Logger” dialog box will appear. Unplug the logger from the PC interface cable and select “OK”
- The “Save As” dialog box will appear. Select the subdirectory (folder) in which to save the data file and enter a file name. The file name should include the 4-letter park unit code, site name, station label (A or C), data type (temp) and monitoring period. Use underscores to separate codes for legibility in the file name, e.g. “CACO_DH_A_temp_July04-05”
- Select “Save” to save the data in the file/folder identified as a *.dtf file for viewing within BoxCar software.
- Export the data to an Excel file by selecting “Export” and “Microsoft Excel Spreadsheet” on the File menu for additional analysis. Use the same naming convention for the data file.
- Repeat this process for the remaining temperature logger.
- Record the date, time, and file names of downloaded data on the Temperature Sensor Deployment Log.

Quadrat Photograph Management

Digital quadrat photographs should be downloaded as soon as possible after they have been taken to ensure accurate photo identification. If a quadrat label was not included in the photos, then all photos must be related to quadrats using field notes. Name each photo file with the 4-letter park unit code, site name, cross-transect, quadrat, and subquadrat/view if applicable (i.e. in instances where water depth did not allow vertical photographs of the entire quadrat, label vertical subquadrat photos “a” – “d” in the order taken and label the oblique full quadrat photo “e”). e.g., CACO_PB_A1, CACO_DH_C2a. File properties should include the photo date, but the files should also be organized into folders named by site and date.

Field Equipment Clean Up

The corrosive properties of salt water and damaging effects of salt crystals can shorten the life of your field gear. Therefore, it is very important to rinse all sampling equipment in fresh water after use. This is also the best time to inventory sampling supplies and equipment and replace any items that will be needed for the next monitoring period.

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